p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, June 2023, 46(2):201-210
DOI: https://doi.org/10.5398/tasj.2023.46.2.201
Available online at http://journal.ipb.ac.id/index.php/tasj

Growth, Health, and Carcass Traits of Broilers Supplemented with *Acalypha australis* L. Leaf Extract, Whey Protein, or their Combination in the Diet

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(Received 13-10-2022; Revised 09-12-2022; Accepted 11-01-2023)

ABSTRACT

The study aimed to investigate the effect of dietary administration of *Acalypha australis* L. leaf extract, whey powder, or a combination of both on the growth, physiological condition, and carcass traits of broilers. A total of 392 broiler chicks was divided into four groups based on a fully randomized design, including (1) control (C, basal feed without additives), (2) basal feed supplemented with 1% *Acalypha australis* L. leaf extract (AALE), (3) basal feed with 1% whey powder (WHEY), and (4) basal feed with 1% *Acalypha australis* L. extract and 1% whey powder (AALEWHEY). Samples (i.e., internal organs, blood, intestinal digesta, small intestinal segments, and breast and thigh meats) were collected on day 33. Data were treated with an analysis of variance followed by Duncan's multiple analysis. Treatments did not affect body weight, feed intake, and feed conversion ratio ($p>0.05$). Abdominal fat was higher in the treated broilers than in the control (C) ($p<0.05$). The serum high-density lipoproteins (HDL) were lower in AALE, WHEY, and AALEWHEY groups than in the control (C) ($p<0.05$). Serum creatinine concentrations were higher in WHEY and AALEWHEY groups than in C and AALE groups ($p<0.05$). The AALE, WHEY, and AALEWHEY groups had higher duodenal villi height than C group ($p<0.05$). The villi height to crypt depth ratios were higher in the jejunum of the WHEY and AALEWHEY group than in the C group ($p<0.05$). The pH of breast meat was higher in the C group than in AALE and AALEWHEY groups ($p<0.05$). The lightness values of breast meats were lower in the AALEWHEY group than in the other groups ($p<0.05$). The redness values of breast meats were lower in the C group than in the AALE, WHEY, and AALEWHEY groups, while the yellowness values were lower in C and AALE groups than in WHEY and AALEWHEY groups ($p<0.05$). The pH values of thigh meats were higher in the AALE group than in control (C), WHEY, and AALEWHEY groups ($p<0.05$). In conclusion, the treatment of broilers with leaf extract of *Acalypha australis* L., whey powder, or a combination of both improved intestinal morphology and meat quality without affecting the growth performance of broilers.

Keywords: antioxidant; broilers; herbs; meat quality; whey

INTRODUCTION

The ban on the use of antibiotic growth promoter (AGP) in feed has been attributed to slower growth rates and a rise in disease prevalence in broiler production. This circumstance has prompted farmers to search for effective AGP substitutes to maintain the sustainability of broiler farming. To increase production efficiency and the health of broiler chickens, farmers frequently employ herbal ingredients as an alternative to AGP. Herbal products contain various active compounds, including antioxidants, immunostimulants, and antimicrobials which can act as anti-stress, boost immune response, and promote the growth of beneficial bacteria while inhibiting the growth of pathogenic bacteria (Sapsuha *et al.,* 2021). One of the herbal plants with phenolic compounds that can serve as antioxidants is *Acalypha australis* Linn leaf (Fan *et al.,* 2012). Gallic acid, which acts as a potent antibacterial agent, is also abundant in *Acalypha australis* L. leaf (Xiao *et al.,* 2013). Additionally, the leaves of *Acalypha australis* L. contain alkaloids, saponins, and flavonoids that are beneficial for enhancing the host's immune system (Kasrina & Zukmadini, 2021; Kim *et al.,* 2020).

Whey is a co-product of the cheese-making process containing a high amount of protein (35% of the dry matter is crude protein). In addition to being a great source of amino acids for chicken nutrition, whey contains bioactive proteins such β-lactoglobulins, α-lactalbumin, and immunoglobulins (Szczurek *et al.,* 2013). Currently, whey is frequently used to enhance chicken health and growth. Pineda-Quiroga *et al.* (2018) revealed that adding whey to feed can improve the microbial community in the broiler intestines while also accelerating growth, and improving mineral digestibility and feed consumption. In line with this, Tsiouris *et al.*

(2020) reported that using whey in feed can improve the growth performance and gut health of broiler chickens. However, whey should not be used excessively in broiler feed since using more than 2% of it has a negative effect on the growth rate of broiler chickens. The ability of broilers to utilize nutrients is negatively impacted by the high concentration of lactose, sugar, and minerals in the whey, which is likely to result in lactose intolerance and intestinal disorder in the broiler (Tsiouris *et al.,* 2020). Note that poultry is lactase deficient, so excess lactose will result in lactose intolerance. Moreover, excess minerals can increase the pH (more neutral) of the intestine, thereby encouraging the growth of pathogenic bacteria. In addition, some minerals, such as Ca, are needed for the synthesis and activity of enterotoxins, so excess minerals can cause damage to the intestinal lining (Paiva *et al.*, 2014).

It is well known that the bioactive components of plant extracts are unstable when stored and delivered to the gastrointestinal tract (Sugiharto, 2021). This can reduce its effectiveness as a phytobiotic for broilers. Therefore, efforts to increase the bioavailability and delivery of plant extracts are crucial. The bioactivity of polyphenols in the body can be increased by combining the polyphenols with whey. Tazeddinova *et al*. (2022) pointed out that the interaction between whey protein and polyphenols can occur during food processing or digestion and may subsequently impact the host's health outputs. Considering the high content of phenolic compounds in *Acalypha australis* L. leaf and the potential interaction between whey protein and polyphenols from the herbs, the *Acalypha australis* L. leaf extract was combined with whey protein and administrated into broiler diets. The present study aimed to investigate the effect of dietary supplementation of *Acalypha australis* L. leaf extract, whey protein or a combination of both on the growth, physiological condition, and carcass traits of broilers.

MATERIALS AND METHODS

Ethical Approval

The Animal Ethical Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, approved the *in vivo* study with approval number 58-04c/A-6/KEP-FPP.

Acalypha australis L. Leaf Extract Preparation

The *Acalypha australis* L. leaves were collected from gardens around the campus of Universitas Diponegoro, Semarang, Central Java Province, Indonesia. After collection, the leaves were air dried and stored at room temperature until extraction. The dried leaves were ground to a fine powder and extracted using 70% ethanol with a ratio of leaf powder: solvent 1:6 (g:mL; Karimy *et al.,* 2013). In brief, dried *Acalypha australis* L. leaf powder was soaked in 70% ethanol for 72 hours at room temperature and then filtered through filter paper to obtain the filtrate. The filtrate was powdered with a vacuum rotary evaporator at a maximum temperature of 60 °C to obtain a paste form (Vongsak *et al.,* 2012). The next step was the granulation process using maltodextrin in a ratio of 25:75 (g:g). After freeze drying, pulverizing was carried out followed by sieving to produce *Acalypha australis* L. leaf extract (in powder form). The extract was stored in a cool room until used for *in vivo* experiment. The *Acalypha australis* L. leaf extract contained a total flavonoid of 675.6 mg/100 g, total phenol of 750.4 mg/100 g, and antioxidant activity of 80.4% inhibition of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS).

In Vivo Study

The study was conducted on 392 Cobb (male+female mixed-sex) broiler chicks and was set up based on a fully randomized design. The chicks were individually weighed (average body weight of 46.29 ± 0.54 g) and then grouped into four dietary treatment groups. Each treatment group consisted of seven replicates/pens containing 14 chickens. The groups were (1) control (C, basal feed without additives), (2) basal feed supplemented with 1% *Acalypha australis* L. leaf extract (AALE), (3) basal feed supplemented with 1% whey powder (WHEY), and (4) basal feed supplemented with 1% *Acalypha australis* L. extract and 1% whey powder (AALEWHEY). The chicks were grown on commercial pre-starter feed containing (according to the feed label) 22%-24% crude protein, less than 5% crude fiber, 5% crude fat, and 7% ash upon arrival until day 7. Formulated starter and finisher feeds (Table 1) were then offered *ad libitum* to the chicks from days 8 to 33. The additives were supplemented to the feed throughout the study period (days 1-33). The study was conducted for 33 days with the consideration that the harvesting age for broiler chickens, in general, is 30-35 days (Liani *et al.,* 2021). The whey powder used in this study was a commercial whey protein powder (80% protein; Davisco Foods International, Le Sueur, Minnesota, USA).

The birds were raised in a broiler house with opened-sides and a litter of rice husks. During the experiment, the lighting program was applied constantly. Chickens were vaccinated against Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) immediately after hatching using the spraying method. At the age of 11 days, the chicks were vaccinated using the Gumboro (infectious bursal disease virus/IBDV) (Medivac Gumboro B vaccine) and on the 18th day, the chickens were also vaccinated with the NDV vaccine (Medivac ND La Sota) through drinking water. Feed consumption and body weight were measured weekly. On day 33, blood was obtained from the wing vein of male chicks (one per pen/replicate; seven per treatment group). Blood was placed in a vacutainer without an anticoagulant. The chickens taken for blood were then slaughtered, and the intestines and internal organs were removed immediately. Segments (~2 cm) of the duodenum, jejunum, and ileum were placed in 10% buffered formalin (Leica Biosystems Richmond, Inc., Richmond, USA) for evaluation of small intestinal morphology (villous height and crypt depth). Digesta from the ileum

Table 1. Feed compositions of broilers

Ingredients (%, unless otherwise noted)	Starter (day 8-21)	Finisher (day 22-33)
Yellow maize	53.50	58.54
Palm oil	2.32	2.96
Soybean meal	40.13	34.70
DL-methionine	0.19	0.19
Bentonite	0.75	0.75
Limestone	1.00	0.75
Monocalcium phosphate	1.30	1.30
Premix[1]	0.34	0.34
Chlorine chloride	0.07	0.07
Salt	0.40	0.40
Chemical compositions		
ME (kcal/kg)[2]	2,900	3,000
Crude protein, %	22.00	20.00
Crude fiber, %	5.47	5.51
Ca, %	1.14	1.02
P (available), %	0.57	0.58
Met, %	0.52	0.49
Lys, %	1.19	1.05

Note: [1]Per kg of feed contained 1.100 mg Zn, 1.000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1.225 mg K, 1.225 mg Mg, 1.250.000 IU vit A, 250.000 IU vit D3, 1.350 g pantothenic acid, 1.875 g vit E, 250 g vit K3, 250 g vit B1, 750 g vit B2, 500 g vit B6, 2.500 mg vit B12, 5.000 g niacin, 125 g folic acid, and 2.500 mg biotin. [2]ME (metabolizable energy) was calculated based on formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}.

and cecum was collected to determine the population of selected bacteria in the intestine. The internal organs were weighed (empty condition) afterward. Samples of breast and thigh meats were obtained for the physical quality (pH and color) assessment of the meats. The coagulated blood was left at room temperature for about 2 hours before being centrifuged at 3.000 rpm for 10 minutes to produce serum. Serum was stored in a freezer (at -10 °C) until analysis.

Laboratory Analysis

Serum lipid profiles (total triglycerides, total cholesterol, low-density lipoprotein [LDL], and high-density lipoprotein [HDL]) and serum uric acid and creatinine levels were determined using enzyme-based colorimetric techniques. The levels of serum total protein, albumin, glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined by spectrophotometric/photometric methods. The serum albumin value was subtracted from the total protein value to calculate the globulin concentration. All biochemical analyses of serum samples were performed according to the manufacturer's instructions (DiaSys Diagnostic System GmbH, Holzheim, Germany). Antibodies towards IBV, IBDV, and NDV were determined at 33, 22, and 15 days, respectively, after vaccination with the respective vaccines. Based on the IDEXX laboratory procedure (USA), the enzyme-linked immunosorbent assay (ELISA) test was conducted to measure antibody levels against IBV and IBDV vaccines. Prior to testing, the antigen-coated plates and IBDV/IBV antibody-ELISA kit were left at room temperature. Chicken serum sample diluent was used to dilute the test sample up to 500-fold. Each well of the plate was then filled with 100 μL of diluted serum samples. The 100 μL of negative control was added to wells A1 and A2, while wells A3 and A4 were given positive controls. Following that, the plate was incubated for 30 minutes at room temperature. Each well was washed (3 times) with 350 μL of distilled water. Each well was poured with a 100 μL dilution of sheep anti-chicken conjugate before 30 minutes of incubation. The washing was then carried out with 350 μL /well 3 times. The Tetramethylbenzidine solution was added to each well in an amount of 100 μL, and the plates were then left to sit at room temperature for 15 minutes. Each well was poured with the stop solution (100 μL) to halt the process. Using the automated IDEXX Diagnostic Software, the absorbance values were then measured, recorded, and calculated. The data are shown as Geometric Mean Titer (GMT). Hemagglutination inhibition (HI) was used to measure the antibodies against NDV, and the results were given as a geometric mean (Log_2). Malondialdehyde (MDA) levels were measured as described by Agusetyaningsih *et al.* (2022). MDA was identified using the thiobarbituric acid (TBA) reactive substance assay.

Small intestinal segments were examined histologically in 5 μm sections of the duodenum, jejunum, or ileum stained with hematoxylin and eosin. The villous height and crypt depth in each segment was determined using an optical microscope equipped with a digital camera (Leica Microsystems GmbH, Wetzlar, Germany). Five measurements were used to calculate the mean values of villous height and crypt depth for each sample. The bacterial population in the ileal and cecal contents was determined based on the total plate count procedure. After 24 h aerobic incubation at 38°C, the number of coliforms and lactose-negative *Enterobacteriaceae* (LNE) were counted as red and colorless colonies on MacConkey agar (Merck KGaA, Darmstadt, Germany). The number of *Enterobacteriaceae* was determined by the total number of coliforms and lactose-negative *Enterobacteriaceae*. After 48 hours of anaerobic incubation at 38 °C, the number of lactic acid bacteria (LAB) was determined on de Man, Rogosa, and Sharpe (MRS; Merck KGaA) agar. To determine the pH value of the meat, 1 g of breast or thigh meat from each sample was homogenized in 9 mL of distilled water, and the pH of the resulting filtrate was measured with a digital pH meter (Hanna Instruments, Woonsocket, Rhode Island). Broiler meat color is measured in Mac OS X with a digital color meter (set to CIE Lab). The values of L* (brightness), a* (redness), and b* (yellowness) are used to represent the color of the broiler meat. Color analysis was performed in triplicate.

Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA, SPSS version 16.0). Duncan's multiple analysis was used when the treatment had a significant effect ($p<0.05$). A p value of 0.05 to less than 0.10 is considered a trend.

RESULTS

Growth Performance and Internal Organ Weight of Broilers

Data on the growth performance of the broiler from day 1 to 33 are presented in Table 2. In general, the effect of dietary treatments was not found on final body weight, body weight gain, feed intake, and feed conversion ratio (FCR) of broilers.

Table 3 shows the relative weights of the internal organs of broiler chickens. It was apparent that abdominal fat content was higher and liver relative weight tended to be lower in the treated as compared to the control chicks ($p<0.05$). The other internal organ weights were not different among the treatment groups.

Antibodies, Serum Biochemical, and Malondialdehyde Levels of Broilers

There is no substantial difference among the treatment groups with regard to antibody titers against NDV and antibodies against IBV and IBDV (Table 4). It was shown that HDL levels were lower in AALE, WHEY, and AALEWHEY groups than in C group chickens ($p<0.05$). The creatinine levels were higher in the serum of WHEY and AALEWHEY groups compared to those in C and AALE groups chickens ($p<0.05$). There was no substantial difference in the levels of other serum biochemical parameters and MDA of experimental broiler chickens (Table 5).

Table 2. Growth performance of broilers supplemented with *Acalypha australis* L. leaf extract, whey protein, or their combination in the diet

Variables	Treatments				SEM	p-value
	C	AALE	WHEY	AALEWHEY		
Day 1-21						
BW (g/bird)	684	702	721	738	9.90	0.247
BWG (g/bird)	638	655	692	675	9.93	0.234
Feed consumption (g/bird)	942	1011	1001	936	18.2	0.345
FCR	1.49	1.55	1.45	1.39	0.03	0.319
Day 22-33						
BW (g/bird)	1530	1535	1557	1500	11.7	0.400
BWG (g/bird)	846	833	819	779	12.2	0.242
Feed consumption (g/bird)	1400	1374	1408	1417	19.4	0.891
FCR	1.67	1.66	1.73	1.83	0.04	0.353
Day 1-33						
BW (g/bird)	1530	1535	1557	1500	11.7	0.409
BWG (g/bird)	1484	1488	1511	1454	11.7	0.410
Feed consumption (g/bird)	2392	2449	2489	2410	27.6	0.632
FCR	1.62	1.65	1.65	1.66	0.02	0.914

Note: C: basal feed without additives, AALE: basal feed supplemented with 1% *Acalypha australis* L. extract, WHEY: basal feed supplemented with 1% whey powder, AALEWHEY: basal feed supplemented with 1% *Acalypha australis* L. extract and 1% whey powder, BW: body weight, BWG: body weight gain, FCR: feed conversion ratio, SEM: standard error of means.

Table 3. Relative internal organ weights of broilers supplemented with *Acalypha australis* L. leaf extract, whey protein, or their combination in the diet

Variables (% live BW)	Treatments				SEM	p-value
	C	AALE	WHEY	AALEWHEY		
Heart	0.48	0.51	0.52	0.53	0.01	0.531
Liver	2.75	2.62	2.40	2.51	0.05	0.072
Proventriculus	0.46	0.45	0.44	0.50	0.01	0.340
Gizzard	1.54	1.59	1.57	1.74	0.04	0.211
Pancreas	0.30	0.30	0.29	0.27	0.01	0.343
Duodenum	0.52	0.62	0.56	0.62	0.02	0.223
Jejunum	1.18	1.11	1.14	1.17	0.04	0.952
Ileum	0.85	0.87	0.77	0.83	0.03	0.751
Ceca	0.41	0.34	0.36	0.42	0.02	0.125
Abdominal fat	0.75^{b}	1.20^{a}	1.13^{a}	1.16^{a}	0.06	0.032
Spleen	0.35	0.14	0.12	0.13	0.06	0.446
Thymus	0.19	0.17	0.18	0.14	0.01	0.542
Bursa of fabricius	0.07	0.07	0.07	0.09	0.01	0.513

Note: ^{a,b}Means in the same row with different superscripts differ significantly ($p<0.05$). C: basal feed without additives, AALE: basal feed supplemented with 1% *Acalypha australis* L. extract, WHEY: basal feed supplemented with 1% whey powder, AALEWHEY: basal feed supplemented with 1% *Acalypha australis* L. extract and 1% whey powder, SEM: standard error of means, BW: body weight.

Selected Bacterial Populations in the Intestines of Broilers

Table 6 shows the numbers of selected bacteria in the intestine of experimental broiler chickens. Both measurements at ileum and caecum at the end of the study showed no significant difference in terms of the number of lactic acid bacteria, coliform, lactose-negative *Enterobacteriaceae*, and *Enterobacteriaceae*.

Morphology of Intestine of Broilers

It was shown in Table 7 that the chicks in AALE, WHEY, and AALEWHEY groups had higher villi height in the duodenum as compared to chicks in the control (C) group ($p<0.05$). Likewise, the ratio of villi height to crypt depth (VH/CD) was higher in the jejunum of the WHEY and AALEWHEY groups when compared especially to that of C group chickens ($p<0.05$).

Carcass Proportions, pH Values, and Color of Broiler Meats

The data on carcass proportion, pH values, as well as the color of the meats of broilers are presented in Table 8. Eviscerated carcass and carcass proportions did not differ among the treatment groups. In breast meat, the pH values were higher in the C group than in AALE and AALEWHEY groups but did not differ from that in the WHEY group ($p<0.05$). The L* values in the breast meat were lower in the AALEWHEY group than in the other groups($p<0.05$). The a* values in the breast meat were lower in the C group than in the other groups, while the b* values of breast meat were lower in C and AALE groups as compared to WHEY and AALEWHEY groups ($p<0.05$). The pH values of thigh meats were higher in the AALE group than in the other groups ($p<0.05$). There was no significant effect of dietary treatments on the color of thigh meats of broilers.

DISCUSSION

Growth Performance and Internal Organ Weight of Broilers

In contrast to the most common impact of herbal products in improving the growth performances of broilers (Hafeez *et al*., 2020; Sapsuha *et al*., 2021), the use of *Acalypha australis* L. leaf extract did not significantly affect the production parameters of broilers in the current study. Our results, however, were consistent with those of Ocak *et al*. (2008) and Yazdi *et al*. (2014), who found no appreciable impact on broiler production parameters when dry peppermint (*Mentha piperita* L.),

Table 4. Antibodies levels of broilers supplemented with *Acalypha australis* L. leaf extract, whey protein or their combination in the diet

Variables	Treatments				SEM	p-value
	C	AALE	WHEY	AALEWHEY		
Antibody titers toward NDV (log_2 GMT)	6.29	6.71	6.43	6.14	0.33	0.942
Antibody toward IBV (GMT)	417	261	627	470	107	0.701
Antibody toward IBDV (GMT)	1378	1651	1825	2110	247	0.782

Note: C: basal feed without additives, AALE: basal feed supplemented with 1% *Acalypha australis* L. extract, WHEY: basal feed supplemented with 1% whey powder, AALEWHEY: basal feed supplemented with 1% *Acalypha australis* L. extract and 1% whey powder, ND: Newcastle disease, IBV: infectious bronchitis virus, IBDV: infectious bursal disease virus, SEM: standard error of means.

Table 5. Serum biochemical and malondialdehyde of broilers supplemented with *Acalypha australis* L. leaf extract, whey protein, or their combination in the diet

Variables	Treatments				SEM	p-value
	C	AALE	WHEY	AALEWHEY		
Total cholesterol (mg/dL)	130	123	109	118	3.68	0.273
Total triglyceride (mg/dL)	56.8	53.9	51.3	59.9	3.69	0.871
LDL (mg/dL)	44.6	49.6	47.1	44.2	2.91	0.910
HDL (mg/dL)	73.9^{a}	62.4^{b}	52.1^{b}	62.1^{b}	2.22	0.023
LDL/HDL	0.63	0.80	0.94	0.71	0.05	0.221
Total protein (g/dL)	2.72	2.69	2.66	2.62	0.06	0.952
Albumin (g/dL)	1.15	1.17	1.17	1.18	0.03	0.991
Globulin (g/dL)	1.57	1.51	1.49	1.44	0.04	0.747
Albumin/Globulin	0.74	0.78	0.79	0.83	0.02	0.213
Uric acid (mg/dL)	3.42	3.04	2.90	2.93	0.19	0.750
Creatinine (mg/dL)	0.04^{b}	0.04^{b}	0.05^{a}	0.05^{a}	<0.01	0.033
AST (U/L)	257	244	260	430	33.5	0.152
ALT (U/L)	0.96	0.91	0.47	0.85	0.14	0.633
MDA (nmol/mL)	2.52	2.58	2.51	2.21	0.12	0.714

Note: ^{a,b}Means in the same row with different superscripts differ significantly ($p<0.05$). C: basal feed without additives, AALE: basal feed supplemented with 1% *Acalypha australis* L. extract, WHEY: basal feed supplemented with 1% whey powder, AALEWHEY: basal feed supplemented with 1% *Acalypha australis* L. extract and 1% whey powder, FCR: feed conversion ratio, LDL: low-density lipoprotein, HDL: high-density lipoprotein, A/G ratio: albumin to globulin ratio, AST: aspartate aminotransferase, ALT: alanine aminotransferase, MDA: malondialdehyde SEM: standard error of means.

Table 6. Selected intestinal bacterial populations of broilers supplemented with *Acalypha australis* L. leaf extract, whey protein, or their combination in the diet

Variables (log cfu/g)	Treatments				SEM	p-value
	C	AALE	WHEY	AALEWHEY		
Ileum						
LAB	3.68	3.36	3.77	3.68	0.29	0.970
Coliform	1.90	1.82	1.33	<1.00	0.21	0.422
LNE	1.64	1.35	1.29	<1.00	0.15	0.541
Enterobacteriaceae	3.68	3.36	3.77	3.68	0.29	0.972
LAB/coliform	2.60	2.46	3.14	3.68	0.29	0.454
Cecum						
LAB	8.06	7.78	7.76	7.33	0.11	0.102
Coliform	6.24	5.84	6.58	5.62	0.21	0.382
LNE	3.74	3.30	3.99	2.71	0.25	0.288
Enterobacteriaceae	6.25	5.84	6.58	5.68	0.20	0.382
LAB/coliform	1.30	1.35	1.19	1.47	0.07	0.561

Note: C: basal feed without additives, AALE: basal feed supplemented with 1% *Acalypha australis* L. extract, WHEY: basal feed supplemented with 1% whey powder, AALEWHEY: basal feed supplemented with 1% *Acalypha australis* L. extract and 1% whey powder, LAB: lactic acid bacteria, LNE: lactose negative *Enterobacteriaceae*, SEM: standard error of means.

Table 7. Small intestinal morphology of broilers supplemented with *Acalypha australis* L. leaf extract, whey protein, or their combination in the diet

Variables	Treatments				SEM	p-value
	C	AALE	WHEY	AALEWHEY		
Duodenum						
Villi height (μm)	1133^{b}	1378^{a}	1340^{a}	1325^{a}	34.8	0.042
Crypt depth (μm)	231	214	200	206	10.6	0.773
VH/CD	5.48	6.67	6.99	6.64	0.33	0.421
Jejunum						
Villi height (μm)	1140	1233	1423	1430	52.4	0.132
Crypt depth (μm)	243	223	203	202	11.5	0.571
VH/CD	4.79^{c}	5.71^{bc}	7.93^{a}	7.32^{ab}	0.41	0.023
Ileum						
Villi height (μm)	590	712	587	639	29.6	0.433
Crypt depth (μm)	124	139	122	133	7.36	0.851
VH/CD	5.07	5.25	5.29	5.42	0.33	0.990

Note: ^{a,b}Means in the same row with different superscripts differ significantly ($p<0.05$). C: basal feed without additives, AALE: basal feed supplemented with 1% *Acalypha australis* L. extract, WHEY: basal feed supplemented with 1% whey powder, AALEWHEY: basal feed supplemented with 1% *Acalypha australis* L. extract and 1% whey powder, VH: villi height, CD: crypt depth, SEM: standard error of means.

thyme (*Thymus vulgaris* L.), or anise seed (*Pimpinella anisum* L.) were added to the diet as supplements. The absent effect of herbal additives on growth performance was also observed by Šťastník *et al*. (2022) when they offered caraway (*Carum carvi* L.) to broiler chickens. Several factors have been reported to cause variations in the effect of herbal ingredients on broiler performance, including the type of herbs, the chemical composition of herbs, the quality of raw herbs, processing methods and conditions, delivery routes, packaging, storage conditions, and herbal dosages (Hafeez *et al*., 2020; Sugiharto, 2021; Šťastník *et al*., 2022). In this investigation, broiler growth performance was found to be unaffected by whey powder supplementation. This result was in line with Ashour *et al*. (2019), who found no effect of whey powder supplementation on the final body weight of broilers. Yet, our finding contrasted with the studies of Pineda-Quiroga *et al*. (2018) and Tsiouris *et al*. (2020), who demonstrated that whey supplementation increased broiler growth rate. The exact reason why whey powder supplementation did not affect broiler growth remains unknown, although using whey at a level of 1% was very likely to interfere with kidney function and hence negatively affected chicken growth in general. Our latter inference was supported by the fact that feeding whey increased the serum concentration of creatinine of broilers in the present study.

It was shown in this study that the proportion of abdominal fat was higher in broilers receiving *Acalypha australis* L. leaf extract, whey or a combination of both when compared with the control broilers. For broiler producers, increasing the proportion of abdominal fat is not profitable as abdominal fat is considered a waste product with no economic value. The precise reason for the increase in the proportion of abdominal fat in broilers receiving *Acalypha australis* L. leaf extract, whey, or a combination of both, compared to control broilers, is unknown. However, the efficacy of *Acalypha australis* L. leaf extract to reduce abdominal fat was probably hindered by the high content of maltodextrin in the product

Table 8. Carcass, pH values, and color of meats of broilers supplemented with *Acalypha australis* L. leaf extract, whey protein, or their combination in the diet

Variables	Treatments				SEM	p-value
	C	AALE	WHEY	AALEWHEY		
EC (% live BW)	66.3	68.3	68.1	67.4	0.39	0.251
Breast (% EC)	35.9	35.4	35.7	34.2	0.43	0.552
Thigh (% EC)	16.8	16.7	15.9	16.5	0.17	0.284
Drumstick (% EC)	14.3	14.9	14.8	14.4	0.22	0.655
Breast meat						
pH	6.48^{a}	6.39^{c}	6.46^{ab}	6.41^{bc}	0.01	<0.01
L*	46.7^{a}	45.6^{a}	45.1^{a}	42.7^{b}	0.39	<0.01
a*	7.25^{b}	10.3^{a}	11.7^{a}	10.7^{a}	0.33	<0.01
b*	14.2^{b}	14.5^{b}	18.8^{a}	19.8^{a}	0.33	<0.01
Thigh meat						
pH	6.63^{b}	6.82^{a}	6.58^{b}	6.65^{b}	0.02	<0.01
L*	43.3	44.1	43.5	44.3	0.33	0.661
a*	14.9	12.1	13.9	13.1	0.48	0.212
b*	13.4	15.4	14.4	15.2	0.32	0.105

Note: ^{a,b}Means in the same row with different superscripts differ significantly ($p<0.05$). C: basal feed without additives, AALE: basal feed supplemented with 1% *Acalypha australis* L. extract, WHEY: basal feed supplemented with 1% whey powder, AALEWHEY: basal feed supplemented with 1% *Acalypha australis* L. extract and 1% whey powder, BW: body weight, EC: eviscerated carcass, L*: lightness value, a*: redness value, b*: yellowness value, SEM: standard error of means.

(the proportion of maltodextrin to *Acalypha australis* L. leaf extract during the granulation process was 75:25 [g:g]). As an energy source, maltodextrin was very likely to increase fat deposition in the abdomen of broiler chickens. Note that increasing dietary energy levels lead to an increase in the activity of some enzymes linked to hepatic lipogenesis resulting in the increased abdominal fat deposition (Fouad & El-Senousey, 2014). In line with this study, Greenhalgh *et al*. (2022) showed that adding whey to the diet increased the proportion of abdominal fat in broiler chickens. The latter investigators inferred that the administration of whey could improve fat digestibility resulting in an increase in fat deposition in the abdomen of broiler chickens. The earlier studies found an association between elevated abdominal fat content and lower serum HDL cholesterol levels in chicks (Després *et al.,* 1988; Musa *et al.,* 2007). In this respect, HDL is responsible for transporting lipids from peripheral tissues to the liver, where the lipids are metabolized. In this study, the lower the HDL level in the blood, the less likely the fat in the abdomen can be degraded by the chicken liver, resulting in high abdominal fat deposits (Després *et al.,* 1988). In this current investigation, the relative liver weight tended to be lower in the treated as compared to the control broilers. A previous study by Vahdatpour *et al*. (2008) showed the inverse relationship between abdominal fat content and liver weight, which is in line with the results of our current study. It was most likely that the supply of lipids from peripheral tissues (including from the abdomen) to the liver was reduced, due to the low serum HDL concentration, causing a decrease in liver fat deposition (Vahdatpour *et al.,* 2008).

Serum Indices of Broilers

It was apparent in this study that HDL level was lower in the chickens receiving either *Acalypha australis* L. leaf extract, whey powder, or a combination of both, when compared with the control chickens. The reason for the low level of HDL in the serum of broilers receiving *Acalypha australis* L. leaf extract, whey powder, or both is not known for certain until now. However, low levels of HDL in serum may be caused by kidney disorders in chickens due to the toxic effect of herbal products or high levels of protein in the feed due to the addition of whey powder. Our inference was in line with Kawachi et al. (2019), who pointed out that low HDL levels can predict kidney disorders in animals. Indeed, the chickens used in this present investigation were believed to experience kidney disorder as indicated by the rise in the levels of creatinine in broilers' serum, particularly in those receiving whey powder or whey powder plus *Acalypha australis* L. leaf extract. Indeed, a rise in blood creatinine levels suggests compromised kidney function in animals (Valchev *et al.,* 2014). With regard particularly to *Acalypha australis* L. leaf extract, the use of herbal products as feed additives was very likely to cause a toxic effect on the kidneys, as reported by Asif (2019) and Daramola (2019). Moreover, a high protein intake brought on by whey powder treatment may result in glomerular hyperfiltration and an increase in intraglomerular pressure. This could exacerbate or develop kidney disorders by damaging the glomerular structure (Ko *et al.,* 2017). However, our present inference should be taken with caution as Ashour *et al*. (2019) showed that feeding whey protein powder up to 0.25% resulted in lower creatinine levels than the control. It is likely that at a low level, whey may improve the health of the broiler kidney (Ashour *et al.,* 2019), while at a high-level, whey (rich in protein) may induce kidney dysfunction (Ko *et al.,* 2017).

Antibody measurements against NDV, IBV, and IBDV did not show any substantial effects of the treatments on broiler chickens in this study. Likewise, lymphoid tissues (spleen, thymus, and *Bursa of fabricius*),

which plays an important role in the immunity of broiler chickens, were also not affected by the treatment of *Acalypha australis* L. leaf extract and whey powder. These present results were not in line with Sugiharto (2021), which shows that the dietary inclusion of herbal products can improve the immune response of broiler chickens. Also, the data in this study do not agree with those reported by Shahsavani *et al.* (2019), in which treatment using whey increased antibody titers against sheep red blood cells (SRBC). Regarding the inconsistency of herbal products on the immunity of broiler chickens, Sugiharto (2021) pointed out that the inconsistency effect of herbal products was largely determined by the nature and dosage of the herbs used and the environmental conditions during the study. Concerning dietary whey treatment, the results in this study were in agreement with those reported by Afkhami *et al*. (2020), in which treatment using whey protein did not affect the humoral immune responses of broilers. Apart from the inconsistent effect of herbal extract and whey on the immune response in broilers, as discussed above, it was also possible that the relatively high temperature (31±1 °C) during the rearing may interfere with the immune response of broilers towards NDV, IBV, and IBDV. This inference was supported by Honda *et al*. (2015), who reported that thermal stress could interfere with the immune responses of chickens toward the ND vaccine.

Malondialdehyde is a marker that is frequently measured to indicate lipid peroxidation brought on by oxidative stress in broiler chickens. In this study, the impact of the experimental treatments was not observed significantly on the MDA level in the chicken serum. In general, the extend of lipid peroxidation due to oxidative stress in broiler chickens varies, greatly depending on the stress conditions experienced by the chickens. Indeed, there is a difference in the effect on lipid peroxidation between acute and chronic stress in broiler chickens. In support of this, Azad et al. (2010) reported that chronic heat stress did not induce lipid peroxidation to a major extent when compared with acute heat stress. One of the stress factors experienced by the chickens during our study period was the hot tropical environmental temperature (31±1 °C). As the chickens continuously experienced high temperatures during the study (thus inducing chronic heat stress on the chickens), these conditions did not seem to induce substantial lipid peroxidation throughout the chicken groups. Such low levels of lipid peroxidation may therefore make the antioxidant activity of *Acalypha australis* L. leaf extract and whey powder not apparently observed.

Intestinal Conditions of Broilers

Morphology of the small intestine shows that dietary treatments of *Acalypha australis* L. leaf extract, whey powder, or a combination of both substantially increased the villous height of the duodenum of broilers. In line with this, the VH/CD was higher in the jejunum of broilers receiving *Acalypha australis* L. leaf extract, whey powder, or their combination. In terms of herbal additives, Farahat *et al*. (2021) reported similar results in which dietary supplementation of plant extract blend (curcuma, chamomile, licorice, and olive leaf) enhanced villi height and VH/CD of the whole small intestinal segments (duodenum, jejunum, and ileum) of broiler chickens. This may lead to the improved intestinal absorption of nutrients and thus growth performance of broilers. In contrast to the latter study, the improved intestinal morphology did not result in the better growth performance of broilers in our current study. As was previously mentioned, the impaired kidney function in the treated broilers may affect physiological circumstances, leading to more energy allocation for maintenance than growth. Regarding whey, the higher villi height of the duodenum and higher VH/CD in the jejunum were in agreement with that reported by Zarei *et al*. (2018). The latter investigators revealed that dietary inclusion of whey powder increased villi height and VH/CD of the small intestine of broilers. Indeed, the improved environment in the intestine for beneficial microbes was attributable to the improved morphology of whey-treated broilers (Ashour *et al.,* 2019). Whey supplementation also lowered the pH values of the small intestine, which may also make the intestinal environment unfavorable for pathogen growth. This can improve intestinal health and lessen damage to the gut mucosa (Zarei *et al*., 2018).

In contrast to the most reported studies (Tsiouris *et al*., 2020; Sugiharto, 2021), the use of herbal ingredients and whey powder did not substantially impact the selected bacterial populations in the gut of broilers in this present study. The microbial population in the intestines of broiler chickens is generally influenced by several factors, including the nutritional content of the feed, nutrient digestibility, hygiene, environmental conditions during the study, etc. With respect particularly to the high tropical environmental temperature, heat stress has been reported to adversely affect the microbial populations in the broiler intestine (Sugiharto *et al*., 2017). In this study, it was very likely that high temperature-induced stress may impair the efficacies of herbal products and whey powder in terms of growth-promoting effect on the beneficial lactic acid bacteria population and the growth-inhibiting effect on pathogenic bacteria. Hence, the effects of *Acalypha australis* L. leaf extract and whey powder were not prominent in this study.

Meat Quality of Broilers

Referring to the earlier studies (Lesiów & Kijowski, 2003; Kralik *et al*., 2017), the chicken meats (both breast and thigh meats) in this study were all included in the dark, firm, and dry (DFD) meats as the pH values of the meats were ≥6.3. This conclusion was also supported by the fact that the meat in this study had a lightness value of <48, which Lesiów & Kijowski (2003) categorized the meat into DFD meat. Prior to slaughter, broilers that have experienced prolonged stress will have less glycogen stored in their muscles. Reduced glycogen levels have an impact on post-mortem metabolism after slaughter, causing the pH to remain high and resulting in the DFD of broiler meat (Kralik *et al*., 2017). During the study, the temperature inside the broiler house (opened-house system) averaged around 31 °C, making it conceivable for broilers to suffer from prolonged heat stress. Apart from the DFD meat characteristics, breast meats of broilers

receiving *Acalypha australis* L. leaf extract, whey powder, or the blend of both showed higher redness (a*) values. Several studies have shown the positive impact of herbal ingredients (Abbasi *et al.,* 2020) and whey (Pineda-Quiroga *et al.,* 2018) on increasing nutrient digestibility, especially protein in broilers. The increase in protein digestibility will increase the availability of protein to be deposited as muscle tissues, which was reflected by the increase in the redness value of the meats. Another study also showed that herbal ingredients (Abbasi *et al.,* 2020) and whey (Afkhami *et al.,* 2020) could improve antioxidant status in broiler chickens. In this regard, *Acalypha australis* L. leaf extract and whey powder seemed to prevent myoglobin denaturation and hence increased the redness values of broiler meats. However, the antioxidative activity of *Acalypha australis* L. leaf extract and whey powder on broiler chickens should be treated carefully, as the levels of MDA were not different across the experimental groups in the present study.

It was shown in this study that the yellowness values of meats were higher in broilers receiving whey powder and a combination of *Acalypha australis* L. leaf extract and whey powder. It has been known that the yellowness values were attributed to the high fat content in the meats (Sirri *et al.,* 2010). Indeed, the chicks receiving whey powder and a combination of *Acalypha australis* L. leaf extract and whey powder had higher fat content in their abdomen, which may also reflect the higher fat content in the meats. In this regard, broilers may store some feed pigments (particularly yellow corn) in fat when their meat has a high fat content (Sirri *et al.,* 2010). Yet, this inference should be taken carefully as the higher abdominal fat content in chicks receiving *Acalypha australis* L. leaf extract did not correspond with the yellowness values of meats.

CONCLUSION

Dietary supplementation of *Acalypha australis* L. leaf extract, whey powder, or a combination of both improved intestinal morphology and meat quality without affecting the growth performance, immune responses, and bacterial populations of broilers. Feeding 1% *Acalypha australis* L. leaf extract, 1% whey powder, or the blend of 1% *Acalypha australis* L. leaf extract and 1% whey powder increase abdominal fat deposition and interfere the kidney functions of broilers. Taken all together, the use of whey powder as much as 1% of feed is recommended for broiler farmers as an alternative to AGP because it is less expensive and commercially available (as compared to *Acalypha australis* L. leaf extract).

CONFLICT OF INTEREST

We declared that we do not have any conflict.

ACKNOWLEDGEMENT

The study was supported by Universitas Diponegoro *via* "Riset Publikasi International" grant, No. 225-24/UN7.6.1/PP/2022.

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